SURFACE-IMMUNOGLOBULIN D OF B-LYMPHOCYTES AND CLOTTING

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SYNOPSIS

Surface-IgD of B-lymphocytes may be important during pregnancy; its absence in mature B-lymphocytes in the retroplacental area may be related to development of immune tolerance. However, pregnancy is a hypercoagulable state. To determine whether clotting has any effect on s-IgD of B lymphocytes, we incubated B-lymphocytes from non-clotted and clotted venous cubital blood samples from ten healthy volunteers with anti-IgD antiserum conjugated with FITC. The mean immunofluorescence was noted in 5.60% ± 3.71 (mean ± standard deviation) for non-clotted samples, and 6.98% ± 4.34 for clotted samples; the difference was not statistically significant. Hence clotting has no effect on s-IgD of B-lymphocytes.

INTRODUCTION

Immunoglobulins on lymphocyte surfaces are of functional significance (1). Surface immunoglobulin D (s-IgD) on B-lymphocytes may be related to lack of tolerance (2). This may be important in the lymphocyte subpopulations around the pregnant uterus; we have reported a diminution of s-IgD on B-lymphocytes from the retroplacental area during pregnancy (3). As blood from the retroplacental area is subjected to an increased coagulation process (4), it is important to determine whether clotting has any effect on the distribution of IgD molecules on B-cell surface.

MATERIALS AND METHOD

Blood (18 ml) was collected from the cubital vein of 5 male and 5 female healthy Chinese volunteers, with a mean age of 23.7 yrs. The females were not pregnant and were not on any form of steroids.

Nine ml. of blood was dispensed into a heparinized (powder) plastic tube and inverted repeatedly. The rest was added into a similar tube but allowed to stand undisturbed for 10 minutes before mixing; a clot is formed in all samples in the upper one-eight of the tube.

Whole lymphocytes were separated on ficoll-isopaque gradients (5). B-lymphocytes enriched population was obtained by rosetting T cells with papain-treated sheep erythrocytes (6). 0.5 x 10^6 washed B lymphocytes were incubated with 40 ul of anti-IgD (fluorescein-conjugated rabbit anti-human IgD, Behringwerke AG) at 1:20 concentration (diluent: medium 1640 with 20% heat inactivated fetal calf serum), at 4°C for 30 minutes. The lymphocytes were fixed in 4% formalin followed by three washes with phosphate buffered saline (PBS) and resuspended in 50% glycerol.

Staining of the lymphocytes was observed with an American Optics Microstar Series One-Ten fluorescence microscope with a halogen source using a FITC fluor cluster. Staining was considered positive only in the presence of obvious and intense fluorescence.

Statistical analyses were carried out with paired t-tests.

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RESULTS

B lymphocytes enriched population which do not form rosettes with sheep RBC constituted 28.09% ± 11.08 (mean ± S.D.) of lymphocytes in male controls, 27.95% ± 5.18 in male clotted samples, 27.99% ± 2.59 in female controls, and 27.26% ± 8.67 in female clotted samples. The ratio is not significantly different between the control and clotted samples.

There is no significant difference in fluorescence s-IgD staining in B lymphocytes between the clotted and control samples in both males (Table 1) and females (Table 2).

DISCUSSION

The results show no significant difference in the T/B ratio of control and clotted samples. Hence, it can be concluded that there has been no differential trapping of either T or B lymphocytes.

The results also show that the initiation of coagulation does not affect the s—IgD of B lymphocytes. This is of relevance in the study of surface immunoglobulins of lymphocytes from the retroplacental area after the delivery of the baby, as it has been demonstrated that labour in associated with hypercoagulability (7).

TABLE 1: B-LYMPHOCYTES STAINED WITH ANTI-IgD ANTISERUM IN CLOTTED AND NON-CLOTTED BLOOD SAMPLES FROM MALE VOLUNTEERS.

Samples	Control			Clotted		
	Total counted	No stained	%	Total counted	No stained	%
CYR	211	4	1.90	248	23	9.27
TKT	200	23	11.50	264	20	7.58
CTH	317	20	6.31	230	15	6.52
CC	95	3	3.16	82	0	0
CYW	136	7	5.15	104	12	11.54
Total	959	57		928	70	
Mean per 100 lympho-cytes (n.s.)	5.60			6.98		
± Standard deviation	± 3.71			± 4.34		

n.s. = statistically not significant

TABLE 2: B-LYMPHOCYTES STAINED WITH ANTI-1gD ANTISERUM IN CLOTTED AND NON-CLOTTED BLOOD SAMPLES FROM FEMALE VOLUNTEERS

Samples	Control			Clotted		
	Total counted	No stained	%	Total counted	No stained	%
KLP	126	9	7.14	99	13	13.13
KH	101	12	11.88	233	28	12.02
AM	207	28	15.53	222	28	12.61
TSH	100	2	2.00	63	2	3.17
CC	178	7	3.93	131	10	7.63
Total	712	58		748	81	
Mean per 100 lympho-cytes (n.s.)	7.70			9.71		
± Standard deviation	± 4.96			± 4.26		

n.s. = statistically not significant

We have demonstrated that s—IgD of retroplacental B-lymphocytes is significantly less than s-IgD of maternal peripheral B-lymphocytes (3). The present study shows that the hypercoagulability has not influenced the interpretation of such results. However, further work is needed to determine the effect on other s-IgD's on both T and B lympocytes.

Gilabert et al have also reported an increase in fibrinogen-degradation products immediately after delivery, implying an increased activity of the fibrinolytic system (8). Tucci, Biagioli and Panero (9) reported that inhibitors of the fibrinolytic system prevented a diminution in s-IgD of fetal lymphocytes after exposure to maternal serum. We are now looking into the relationship between the fibrinolytic system and surface immunoglobulin in lymphocytes during pregnancy.

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